The Effect of Electromagnetic Radiation Transmitted from Routers on Antibiotic Susceptibility of Bacterial Pathogens

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ABSTRACT

Background: Electromagnetic non-ionizing radiation has both thermal and non-thermal outcomes on biological systems, such as humans, animals, and bacteria.

Objective: This study aimed to investigate the effect of non-ionizing radiofrequency radiation, emitted by Wi-Fi routers, on bacterial strains and the modification of their susceptibility to modern antibiotics.

Material and Methods: In this case-control paired study, four bacteria were selected, and one colony from each bacterial strain was exposed to Wi-Fi radiation forming the exposure group. Another set of colonies was not exposed to Wi-Fi radiation, forming the control group. Eight different antibiotic disks were set on the bacterial plates, and the inhibition zone was measured every 3 h for each colony.

Results: Electromagnetic radiation affects bacterial colonies and their susceptibility to antibiotics. Analysis revealed statistically significant differences, correlated with the bacterial strain, the antibiotic agent, and the time of the exposure, in the inhibition zones, mostly after 6 and 24 h (p-value < 0.05).

Conclusion: A correlation was observed between antibiotic susceptibility and non-ionizing radiofrequency exposure. Studying the effects of radiofrequency radiation on prokaryotic organisms could clarify more complicated cell structures and organisms, such as eukaryotic. Further experiments, in vitro and in vivo, could provide more information about these outcomes and cause experts to discuss the current guidelines of exposure limits.

Keywords

Anti-Bacterial Agents; Bacteria; Drug Resistance; Radiofrequency Radiation; Wi-Fi; Wireless Technology

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Introduction

Antibiotic drug resistance is developed into a global phenomenon and a significant threat to public health. Prolonged hospitalization, higher pharmaceutical costs, and increased mortality, especially in intensive care units, are some of the most common consequences [1,2]. Modern antibiotics, designed and developed for the management of serious infections by multi-resistant bacteria, are widely used in common infections due to resistance to front-line medication. Global campaigns for the restriction of oral antibiotics overuse have

been launched for communities to comply with the international guidelines [3,4].

The present experimental protocol investigates the effects of other factors, such as non-ionizing radiation, on antibiotic susceptibility [1,5,6]. Due to the tremendous technological development in recent years, exposure to such kinds of radiation has been dramatically increased, not only in adults but in young children. Wireless communications are a modern trend, leading to technological development. Several studies cause implications regarding the safety of such exposures [7-9].

Non-ionizing radiation, such as microwave and radiofrequency radiation, affects not only humans but other living organisms. In addition to the known thermal effects, significant non-thermal outcomes are on living organisms [10]. Accordingly, the scientific community has also focused on non-thermal outcomes, which are not as obvious as the thermal ones [11-18].

Wireless Fidelity (Wi-Fi) routers, also called "modems", are small stations that provide an internet connection to the devices connected via Wi-Fi Protected Access (WPA-PSK) encryption. A connection can be either wireless or wired, and Wi-Fi technology consists of a "family" of wireless network protocols based on the Institute of Electrical and Electronics Engineers (IEEE 8211) standards. Broadcast frequencies are mainly 2.4 and 5 GHz [19].

Studies have emerged regarding the bacterial resistance to antibiotics, about the exposure to electromagnetic waves. Movahedi et al. revealed that exposure to mobile electromagnetic radiation (RF-EMFs) for a whole day increases the resistance in *Staphylococcus aureus and Pseudomonas aeruginosam* [1]. A case-control experiment revealed that the exposure to Wi-Fi radiation might increase the metabolic activity in *Escherichia coli, Staphylococcus aureus*, and *Staphylococcus epidermis*, implicating a possible augmentation of the resistance [5]. Escherichia Coli was also affected by the electromagnetic radiation, emitted from common Wi-Fi systems [6]. In addition, Taheri et al. showed that Wi-Fi radiation affects the sensitivity of *Klebsiella pneumoniae.* Another study revealed the non-thermal effects of microwaves (MW) on the antibiotic sensitivity of *Pseudomonas aeruginosa* [20].

Routers, installed in houses, public buildings, shops, restaurants, hospitals, open public areas, and public squares, have inundated the lives of people. Wi-Fi function is mostly turned on the whole day; therefore, an electromagnetic cloud is formed from the networks surrounding. Humans, but also microorganisms, are radiated during their abidance inside the "cloud". Antibiotic susceptibility of bacteria exposed to non-ionizing radiation of Wi-fi routers was measured and statistically analyzed to study these effects.

Material and Methods

This case-control paired study was conducted with the laboratory of bio-pathology and microbiology of Hippokratio hospital in Thessaloniki, Greece. According to the literature [1,2,5,6], four microbial strains were selected according to their availability and the already existing data from other research so that the results could be comparable

The selected strains were three Gram (-) bacteria, *Escherichia Coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and one Gram (+) coccus, Staphylococcus aureus. Antimicrobial disks were placed into the cultural plates to measure the antibiotic susceptibility, using the basic methodology elements of the Kirby-Bauer method. The plates were divided into two groups: the control and the exposure group (experimental). The control group was incubated in the central incubation chamber inside a Faraday bag at 37 C° to exclude any radiation exposure. The exposure group was incubated in a subsidiary chamber at 37 C°, in which the source of the radiofrequency radiation (RFR) was mounted 30 cm away.

For both control and exposure groups, stan-

dard turbidity of 0.5 McFarland was swabbed on Muller-Hinton agar to have 1.5×10^8 CFU/ml as the total count. For *staphylococcus aureus* blood agar was used as a nutrient medium. The selected source of RFR was a Wi-Fi router, configured properly for the experiment [1,2,5,6]. The router was designed and developed by Ubiquiti, broadcasting wireless at 5 GHz.

A laptop was connected to the router and exchanged data at 1.6 Mbps during the whole experimental process, which lasted 24 h. The selected method does not compromise with the standards, in which readings of the inhibition zones are done after 18 or 24 h, depending on the pathogen. The current research depends on continuous exposure and the simultaneous measurement of the experimental parameters. Bacterial growth and exposure to non-ionizing radiation increase in parallel time, which was the gold standard of this research. The selected method does not controvert the standard Kirby-Bauer method [21,22], following the same concept as literature; however, it uses a modified script to investigate the null hypothesis. Additionally, the current study aimed to observe and measure the differences produced by the effect of non-ionizing radiation on a biological factor, such as antibiotic susceptibility. Therefore, measurements were performed every 3 h (3^{rd} h, 6^{th} h, 9^{th} h, 12^{th} h, 15^{th} h, and 18^{th} h) except the last one, which was held 6 h after the 18^{th} h (24^{th} h). The antibacterial disks used in the experiment were selected based on the international literature, the bacterial characteristics, and their antibacterial range [1,2,5,6].

The eight antibacterial agents selected were as follows: Piperacillin-Tazobactam (110 µgr), Imipenem (10 µgr), Cefotaxime (30 µgr), Ciprofloxacin (5 µgr), Aztreonam (30 µgr), Cotrimoxazole (25 µgr), Levofloxacin (5 µgr), and Ceftriaxone (30 µgr). These agents were inserted into the culture plates in a set of four disks per plate. The diameter of each disk was standardized to 5mm by the manufacturer; a radial diffusion zone was developed around each disk. The diameter of the zone was proportional to each susceptibility of the pathogen to the agents and also measured with a Vernier caliper in millimeters. Every three hours, the plates were removed from the chambers for the measurements and were then reinserted to continue with the process of incubation.

The radial diffusion zone for each antibiotic disk for both two groups of the four bacteria was computed every three hours.

In this case-control paired study, controls consist of the non-radiated bacteria cultures while the cases consist of the Wi-Fi exposed bacteria. The statistical processing of the results was done by the method of analysis of variance of two factors without interaction and with statistically significant values of variable $p < 0.05$. The t-test was used to determine whether or not two populations are statistically different from each other, by processing the difference in means and variances. The samples are independent of each other and present normal distribution. Statistical analysis was conducted using SPSS (version 24.0, IBM, SPSS Inc., Chicago, IL, USA). P-values under 0.05 were statistically significant.

Results

The radial diffusion zone for each antibiotic disk was calculated in millimeters (Tables 1-5 and Figures 1 and 2). With sign R (resistant), zero measurements of the diffusion zone were recorded as 5 mm (millimeters) equal to the diameter of the disk (Table 1). In the first three hours, no bacterial development was seen for all four studied bacteria, and there were no statistically significant differences to declare (p-value > 0.05). Similar results were presented by other studies, in the first three hours, using different culturing techniques [1,2,5,6]. First signs of bacterial growth were observed at the next checkpoint, six hours from the beginning of the experiment, in which the first results were recorded (Figure 1).

Analyzing the results, a common behavior

Table 1: Bacterial growth observed at each checkpoint from the beginning of the experiment. Experimental radiated group vs. control non-radiated group.

Antibiotic Agent	Escherichia Coli		Klebsiella pneumoniae		Pseudomonas aeruginosa		Staphylococcus aureus	
	*CTRL	*EXP	CTRL	EXP	CTRL	EXP	CTRL	EXP
6 hours								
TZP	22	20	19	16	26	R	20	14
IMI	R	R	R	R	R	R	R	R
LEV	29	26	18	16	15	14	R	R
ATM	26	22	13	10	20	R	R	R
CIP	30	30	21	20	16	14	8	R
CTX	25	24	20	18	14	R	15	10
COT	24	22	18	14	R	R	R	R
CRO	26	22	17	14	15	R	14	12
9 hours								
TZP	23	23	19	18	26	26	25	13
IMI	R	R	R	R	R	R	12	R
LEV	33	32	18	18	22	23	9	R
ATM	28	28	13	13	22	23	R	R
CIP	33	35	22	20	29	25	9	R
CTX	25	28	18	18	17	16	22	19
COT	24	25	15	15	R	R	22	22
CRO	25	28	15	17	18	15	20	18
12 hours								
TZP	24	25	19	17	30	30	25	27
IMI	R	R	R	R	R	R	9	10
LEV	29	35	20	20	21	25	9	7
ATM	30	30	13	12	26	25	R	R
CIP	33	38	21	20	30	29	10	6
CTX	30	29	18	27	19	19	22	22
COT	24	26	15	14	R	R	22	23
CRO	28	30	16	17	21	19	20	19
15 hours								
TZP	24	25	20	18	29	28	27	27
IMI	R	R	R	R	R	R	12	10
LEV	31	35	20	20	22	24	8	7
ATM	29	30	12	13	27	26	R	R
CIP	35	35	21	22	31	30	10	R
CTX	29	28	19	19	20	20	21	21
COT	24	25	13	13	R	R	23	25
CRO	28	30	16	17	21	21	19	19

Antibiotic Agent	Escherichia Coli		Klebsiella pneumoniae		Pseudomonas aeruginosa		Staphylococcus aureus	
	CTRL	EXP	CTRL	EXP	CTRL	EXP	CTRL	EXP
18 hours								
TZP	24	25	20	18	30	30	29	27
IMI	R	6	R	R	7	6	8	10
LEV	30	31	20	21	23	24	7	8
ATM	30	29	13	12	27	27	R	6
CIP	30	31	22	22	30	32	10	7
CTX	29	30	19	18	20	19	20	21
COT	23	25	11	12	6	6	23	25
CRO	30	31	17	17	23	20	18	19
24 hours								
TZP	26	25	20	18	30	30	25	26
IMI	6	6	6	6	6	6	10	9
LEV	31	35	20	20	23	25	8	7
ATM	30	31	14	12	27	26	6	6
CIP	35	32	22	22	30	35	10	6
CTX	30	30	19	17	20	18	20	21
COT	23	23	11	10	6	R	22	24
CRO	30	31	17	16	20	20	19	19

TZP: Piperacillin-Tazobactam (110 µgr), IMI: Imipenem (10 µgr), CTX: Cefotaxime (30 µgr), CIP: Ciprofloxacin (5 µgr), ATM: Aztreonam (30 µgr), COT: Cotrimoxazole (25 µgr), LEV: Levofloxacin (5 µgr), CRO: Ceftriaxone (30 µgr), CTRL: Control Group, EXP: Experimental Group

Table 2: Paired Samples Test *Escherichia coli* (with **bold** are highlighted the statistically significant differences).

		Paired Differences				t	df	Sig. (2-tailed) P-value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper			
Pair1	*CTRL6 - EXP6	2.00000	1.60357	0.56695	0.65938	3.34062	3.528	7	**0.010**
Pair2	CTRL9 - EXP9	-1.00000	1.51186	0.53452	-2.26394	0.26394	-1.871	7	0.104
Pair3	CTRL12 - EXP12	-1.87500	2.47487	0.87500	-3.94405	0.19405	-2.143	7	0.069
Pair4	CTRL15 - EXP15	-1.00000	1.51186	0.53452	-2.26394	0.26394	-1.871	7	0.104
Pair5	CTRL18 - EXP18	-0.87500	0.83452	0.29505	-1.57268	-0.17732	-2.966	7	**0.021**
Pair 6	CTRL24 - EXP24	-0.25000	1.98206	0.70076	-1.90705	1.40705	-0.357	7	0.732

*CTRL#:Control Group #Hour, EXP#: Experimental Group #Hour

Table 3: Paired Samples Test *Klebsiella pneumoniae* (with **bold** are highlighted the statistically significant differences).

		Paired Differences				t	df	Sig. (2-tailed) P-value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper				
Pair 1	*CTRL6 - EXP6	2.25000	1.28174	0.45316	1.17844	3.32156	4.965	7	**0.002**
Pair 2	CTRL9 - EXP9	0.12500	1.12599	0.39810	-0.81635	1.06635	0.314	7	0.763
Pair 3	CTRL12 - EXP12	-0.62500	3.50255	1.23834	-3.55321	2.30321	-0.505	7	0.629
Pair 4	CTRL15 - EXP15	-0.12500	0.99103	0.35038	-0.95352	0.70352	-0.357	7	0.732
Pair 5	CTRL18 - EXP18	0.25000	1.03510	0.36596	-0.61536	1.11536	0.683	7	0.516
Pair 6	CTRL24 - EXP24	1.00000	0.92582	0.32733	0.22600	1.77400	3.055	7	**0.018**

*CTRL#:Control Group #Hour, EXP#: Experimental Group #Hour

Table 4: Paired Samples Test *Pseudomonas aeruginosa* (with **bold** are highlighted the statistically significant differences).

		Paired Differences				t	df	Sig. (2-tailed) P-value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper				
Pair 1	*CTRL6 - EXP6	7.25000	7.85130	2.77585	0.68615	13.81385	2.612	7	**0.035**
Pair 2	CTRL9 - EXP9	0.75000	1.83225	0.64780	-0.78180	2.28180	1.158	7	0.285
Pair 3	CTRL12 - EXP12	0.00000	1.77281	0.62678	-1.48211	1.48211	0.000	7	1.000
Pair 4	CTRL15 - EXP15	0.12500	0.99103	0.35038	-0.70352	0.95352	0.357	7	0.732
Pair 5	CTRL18 - EXP18	0.25000	1.48805	0.52610	-0.99404	1.49404	0.475	7	0.649
Pair 6	CTRL24 - EXP24	-0.37500	2.19984	0.77776	-2.21411	1.46411	-0.482	7	0.644

*CTRL#:Control Group #Hour, EXP#: Experimental Group #Hour

for all four bacteria was observed related to the divergence of antibiotic susceptibility. The maximum disparity was observed at the same checkpoint for all bacteria, at the logarithmic phase of their growth, the 6th hour. A stabilization was observed in the recorded differences and, in some cases, an inversion of the results among the control and exposure groups (Table 1).

The paired difference is presented for each of the studied pathogens. The comparison was done among each pair (Control – Experimental) for all the antibiotic agents tested, at every checkpoint.

The variation of the susceptibility with notable differences of four selected antibiotic agents against each bacterial strain is presented below (Figure 2).

Discussion

From the recorded results during the experiment, a reduction in antibiotic susceptibility

Table 5: Paired Samples Test *Staphylococcus aureus* (with **bold** are highlighted the statistically significant differences).

	Paired Differences					t	df	Sig. (2-tailed) P-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1	*CTRL6 - EXP6	2.00000	2.44949	0.86603	-0.04782	4.04782	2.309	7	**0.054**
Pair 2	CTRL9 - EXP9	4.00000	3.96412	1.40153	0.68591	7.31409	2.854	7	**0.025**
Pair 3	CTRL12 - EXP12	0.37500	1.92261	0.67975	-1.23234	1.98234	0.552	7	0.598
Pair 4	CTRL15 - EXP15	0.75000	2.05287	0.72580	-0.96624	2.46624	1.033	7	0.336
Pair 5	CTRL18 - EXP18	-0.37500	1.84681	0.65295	-1.91897	1.16897	-0.574	7	0.584
Pair 6	CTRL24 - EXP24	0.25000	1.83225	0.64780	-1.28180	1.78180	0.386	7	0.711

CTRL: Control Group, EXP: Experimental Group

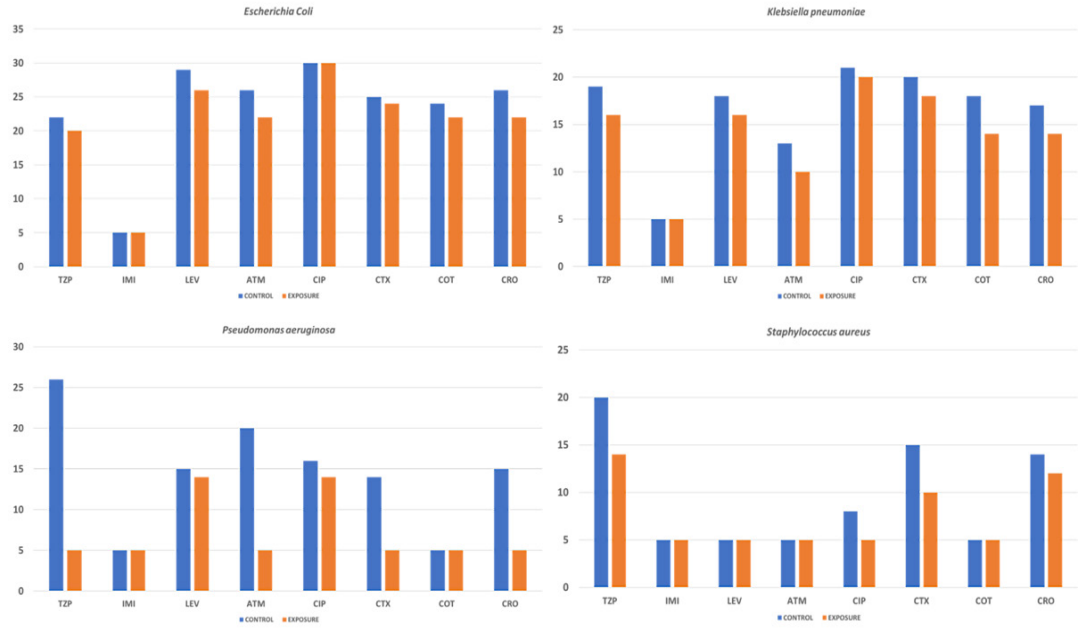

Figure 1: Comparisons of the bacterial growth observed six (6) h from the beginning of the experiment. Exposed group vs. control group. The x-axis represents the 8 antibiotic agents selected and Y-axis stands for the mean inhibition zone of each pathogen.

was observed. The major difference was recorded mainly during the logarithmic phase of the bacterial growth [1,5,6,18]. More specifically, at the sixth hour, *E. Coli* presented a p-value of **0.010**, *Klebsiella pneumoniae* **0.002**, *Pseudomonas aeruginosa* **0.035**, and *Staphylococcus aureus* marginally significant at **0.054**. These findings support our hypothesis regarding the alterations in the susceptibility due to the Wi-Fi RFR. A significant decrease

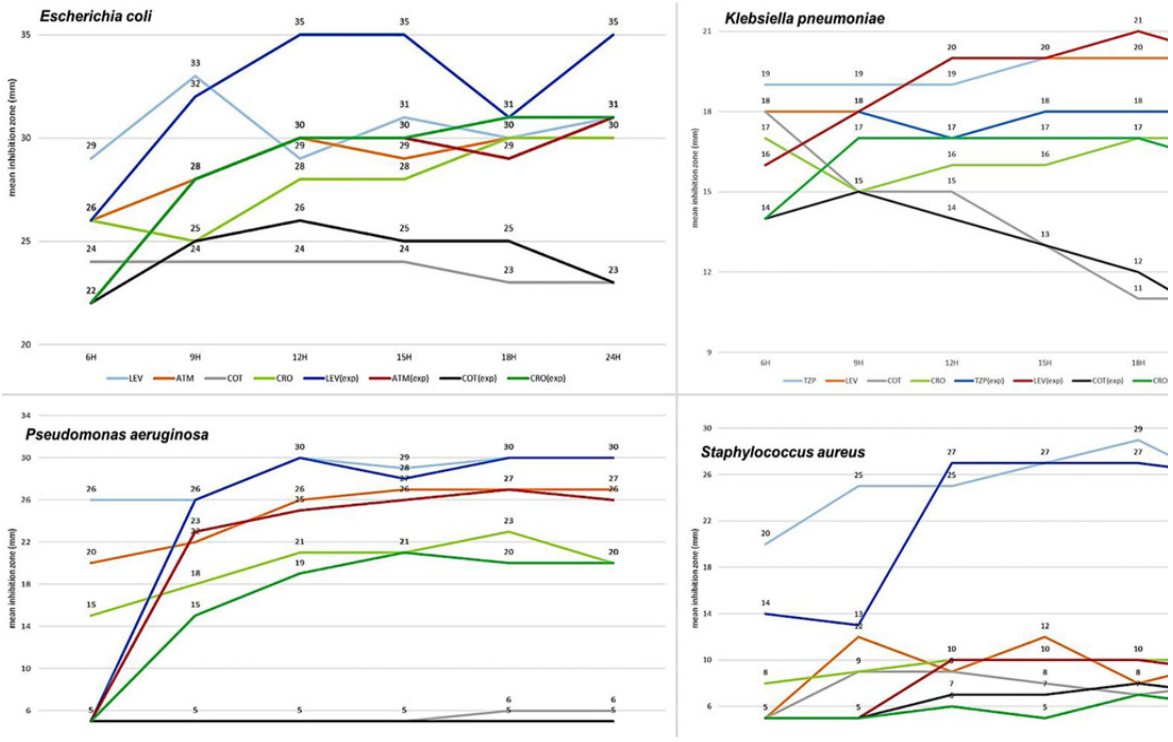

Figure 2: Mean inhibition zone diameter in the studied strains against four selected antibiotics during 24 hours.

in the inhibition zone diameters was observed, showing an antibacterial resistance pattern due to the radiation. The next checkpoints presented fewer disparities, until the 24th h. These results were similar to other studies [2,6,20].

In this study, the checkpoints were set at 3, 6, 9, 12, 15, 18, and 24 h with the first recorded results at 6th h. A notable difference was observed at the beginning of the logarithmic phase (6 h), which constituted the first recorded result. Three hours after the beginning of the experiment, no growth was observed at any of the culture plates. In other studies, results are recorded much earlier in four or even two hours from the beginning of the experiment [1,2]. In the current study, based on a team of biopathology experts, the time needed for the first results to emerge, was similar to the usual time that a bacterial culture needs to reach the logarithmic phase.

The analysis revealed two facts as follows: 1) the strong correlation between time and antibiotic susceptibility [3] and 2) the recorded significant differences (p-values < 0.05) in our four pathogens, at the second checkpoint. Significant findings (p-values < 05) were recorded also in other checkpoints (*E. Coli* at the 18th hour, *Klebsiella pneumoniae* at the 24th h, and *Staphylococcus aureus* at the 9th h); however, the observations at the 6th h pertained to all pathogens (Table 1).

The variation of the antibiotic susceptibility under the effect of RFR is a common finding in several published studies [1,2,5,18]. This susceptibility variation is less for bacteria exposed to RFR. These mechanisms are related not only to the structure of the bacterial membrane and the bacterial wall but also to their biochemical properties. Electromagnetic waves may also affect the potential of the bacterial wall and the ions exchange. The molecules of water present in the bacterial cell are bipolar. Therefore, inside a magnetic field, these molecules are orientated towards

the magnetic lines. Molecules and bigger substances are transferred in and out of the bacterial cell via bacterial membranous proteins. The structure of these proteins may be altered under the effect of an electromagnetic field [6,15]. Therefore, some researchers also examine the effect of these alterations on the structure of antibiotics.

An important role in all of these mechanisms plays the intensity and the type of radiation. However, many disparities are based on the sensitivity of the experimental conditions [6,15,17].

A similar investigation focused on Pseudomonas aeruginosa and *Staphylococcus aureus*, revealed that exposure to electromagnetic waves at a frequency of 2.4 GHz, leads to the augmentation of the bacterial resistance against them, especially after 24 h [23]. In the current study, *Pseudomonas aeruginosa* resistance was increased for the cases of Cefotaxime, Aztreonam, and Cotrimoxazole, 24 h after the exposure (Table 1), which is following the aforementioned study [23]. Moreover, S. aureus resistance was increased for the cases of Imipenem, Ciprofloxacin, and Levofloxacin, similarly to the above study [23].

In the present study, many of the given drugs led to resistance 24 h after the exposure, especially, for *Klebsiella pneumoniae* with a full-spectrum resistance. Future studies aimed to focus on the development of further research protocols and increase the number of statistical samples. The experience gained from this study indicates that the bigger the sample can lead the safer the conclusions. Therefore, future research should also study other types of RFR and compare the results to exclude the factor of randomness.

Numerous studies just justify the growing concerns regarding the non-ionizing EMF that correlated with the development of malignant tumors in rats [24]. Apart from the risk of cancer, EMF could be responsible for "Microwave Syndrome", known as "electromagnetic hypersensitivity" (EHS), a phenomenon characterized by the appearance of symptoms after exposure of people to electromagnetic fields. These symptoms are generally non-specific multiple organ symptoms that manifest in the skin and nervous system, respiratory, cardiovascular, and musculoskeletal systems. Central nervous system symptoms are the most common as a consequence of the neural damage and the over-sensitized neural responses due to RF-EMFs [25,26]. The upcoming 5G mobile network might have adverse systemic outcomes due to the synergistic effects of other toxic stimuli [27]. The accumulative outcomes are directly linked to deoxyribonucleic acid (DNA) damage, leading to cancer, neurodegenerative diseases, and reproductive declines [28].

Investigation of the RFR effects on biological systems is vital. The present study focused on the drug-resistance phenomena due to RF-EMFs and showed a correlation between antibiotic susceptibility and RFR.

However, this study aimed to investigate the non-thermal effects of RFR, the temperature rise during the irradiation can be an important parameter. However, it was not feasible to measure the temperature rise due to the special conditions of our specimens' storage inside the incubation chambers, the culture chambers were regulated to hold a specific temperature that the bacteria needed (37o C during the experiment in both exposure and control group).

Additionally, it was impossible to measure the specific absorption rate (SAR) due to the nature of the experimental specimens. The experiment was based on bacterial strains with very limited mass; therefore, the SAR could not be calculated.

Conclusion

Antibiotic susceptibility and non-ionizing radiofrequency exposure correlated. The effects of radiofrequency radiation on prokaryotic organisms could clarify more complicated cell structures and organisms, such as eukaryotic. Further experiments, in vitro and in vivo,

could provide more information about these outcomes and arise experts for further discussion on the current guidelines of exposure limits.

Authors' Contribution

A. Pegios and E. Vagdatli conceived the idea. The introduction of the paper was written by A. Pegios and D. Kavvadas. A. Pegios, D. Kavvadas, and K. Zarras gathered the images and the related literature and also help with the writing of the related works. The method implementation was carried out A. Pegios, K. Mpani, P. Soukiouroglou, and S. Charalampidou. Results and analysis were conducted by K. Zarras, A. Pegios, and D. Kavvadas. The research work was proofread and supervised by T. Papamitsou. All the authors read, modified, and approved the final version of the manuscript.

Ethical Approval

Permission for conducting the experiment was granted from the Hippokratio General Hospital of Thessaloniki, Greece.

Conflict of Interest

None

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